Abstract of the Disclosure

Methods and kits of the present invention provide an optimized system for cDNA microarray analysis. These systems include novel labeling, hybridization and stringency wash methods and compositions. These novel methods and kits provide robust and cost-effective protocols for labeling target cDNA for hybridization to probe nucleic acid immobilized onto a solid substrate, for highly efficient hybridization of target cDNA to probe nucleic acid, and for detection/visualization of these hybridizations wherein zero or almost zero background signal (*i.e.*, "noise") is generated. The high quality and sensitivity of the hybridization reactions thus provides statistically robust data for gene expression analysis.

In one embodiment, methods for labeling target cDNA utilize a labeling mixture comprising 48μM dATP, 48μM dCTP, 48μM dGTP, 6μM dTTP and 6 μM of fluorescently labeled nucleotide selected from the group consisting of dUTP-Cy3™ and dUTP-Cy5™, and further utilize the Klenow fragment of DNA polymerase I to produce labeled target cDNA. In another embodiment, target cDNA that has been labeled according to the unique labeling mixture is hybridized to a microarray comprising a plurality of probe nucleic acid samples, wherein the microarray has been treated with a pre-hybridization buffer comprising 5X SSC, 1 % BSA Fraction V and 0.1% SDS, where the SDS has a pH of between about 7.18 and about 7.25. In another embodiment, target cDNA and probe nucleic acid immobilized onto a microarray are hybridized in a buffer comprising polyA RNA, Calf Thymus DNA, 5X SSC, 5X Denhard's solution, 50% formamide, and 0.5% SDS, wherein the SDS has a pH of between about 7.18 and about 7.25. In another embodiment, a microarray comprising labeled target cDNA hybridized to probe nucleic acid is washed at least three times with post-hybridization buffers, wherein the first buffer comprises 1X SSC and 0.2% SDS, the second buffer comprises 0.1X SSC and 0.2% SDS, and the third comprising 0.1 % SSC, wherein the SDS has a pH of between about 7.18 and about 7.25.